Plant Epigenetics and Crop Improvement

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Contents

Abstract

Developmental cues and environmental signals remodel the chromatin structure, thus affecting various processes, including flowering time, imprinting, floral development, and biotic and abiotic stress responses in plants. Chromatin remodeling through histone tail post-translational modifications, DNA methylation, and ATP-dependent nucleosome reorganization represents a ubiquitous mechanism to regulate gene expression. Most of the epigenetic and epigenomic studies for the regulation of gene expression in response to developmental and environmental stimuli have been carried out in *Arabidopsis*. Although genetic modifications have been used for crop improvement, however, the epigenetic modifications are at their beginning. In this chapter, we summarize the roles of chromatin-remodeling mechanisms in response to environmental stimuli and discuss their potential for crop improvement.

Keywords

Histone modifications • Chromatin remodeling • DNA methylation • Biotic and abiotic stresses • Crop improvement

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Introduction

In eukaryotic cell nuclei, genomic DNA is packaged into a highly organized nucleoprotein complex known as chromatin. The fundamental unit of chromatin is the nucleosome, which is composed of ~147 base pairs of DNA wrapped around a core of eight histone molecules (two copies of each of the histones H2A, H2B, H3 and H4). Nucleosomes are not simply static structural units, but are rather dynamic. Nucleosomes can be moved, stabilized/destabilized, and disassembled/reassembled at particular genome locations in response to specific environmental signals or developmental cues. The resulting dynamic of the chromatin structure directly modulates the DNA accessibility, thus regulating all DNA-template processes (i.e., transcription, DNA replication, DNA repair, recombination, transposition, or chromosome segregation) and affecting various processes in plants such as root growth, flowering timing, floral organogenesis, gametophyte or embryo formation, as well as the response to pathogens or environmental changes (Berr et al. 2011). However, not all genes are active at all times. Therefore, cells use several mechanisms along the genome to alter the chromatin structure and the properties of a nucleosome in order to specifically control gene expression. Regulation of gene expression within the chromatin context is controlled by different mechanisms, including nucleosome assembly, ATP-dependent nucleosome reorganization, DNA methylation, and post-translational covalent histone modifications (e.g., acetylation, ubiquitination, methylation, phosphorylation, sumoylation).

Different epigenetic regulators are controlling all the above mechanisms, and the changes in these regulators can influence gene expression of a particular gene or set of genes, while the underlying DNA sequence remains identical (Jablonka and Raz 2009). Most of these changes are reversible developmental effects, and they are part of molecular processes encoding phenotypic plasticity in response to environmental variation (Richards et al. 2010) (Fig. 1). However, inheritable chromatin changes have also been reported (Jablonka and Raz 2009). At this point, it is important to clarify that those modifications which are not inheritable are not included in epigenetics as according to the definition of epigenetics, these modifications must be inheritable (mitotic and/or meiotic). Thus, we can broadly classify these modifications into nonheritable chromatin modifications (chromatin modifications that are the result of processes such as DNA repair or phosphorylation of serine 10 of histone H3, which are observed only at specific times during the cell cycle and are, therefore, unlikely to encode epigenetic information (Springer 2013)) and heritable chromatin modifications. The heritable chromatin modifications can further be classified into mitotically transmissible modifications that are reset in the next generation and meiotically transgenerational chromatin modifications that are inherited/transmitted to the following generations. The mitotically stable epigenetic marks, which accompany development, are mainly histone modification, but there are some examples of involvement of DNA methylation as well (Lauria et al. 2004; Zemach et al. 2010; Khan et al. 2013). However, DNA methylation can exhibit a relatively stable pattern of inheritance even over hundreds of years (Cubas et al. 1999; Manning et al. 2006). Because heritability determines the potential of changes or variations of a trait, it is essential to determine the degree of heritability of epigenetic modifications, their impact on given ecologically important traits (Fisher 1930; Falconer 1996), their role in individual adaptation to changing environment (Visser 2008; Hoffmann and Sgrò 2011) and ultimately in crop improvement.

The heritable epigenetic mutations, i.e., epimutations/epialleles, can be classified into three categories on the basis of relative dependence on the genotype. Pure epialleles constitute the first category, which is solely epigenetic, meaning that they are independent of the genetic variations. The second category is facilitated epialleles, which are not fully dependent on genetic variation, although they are linked and even

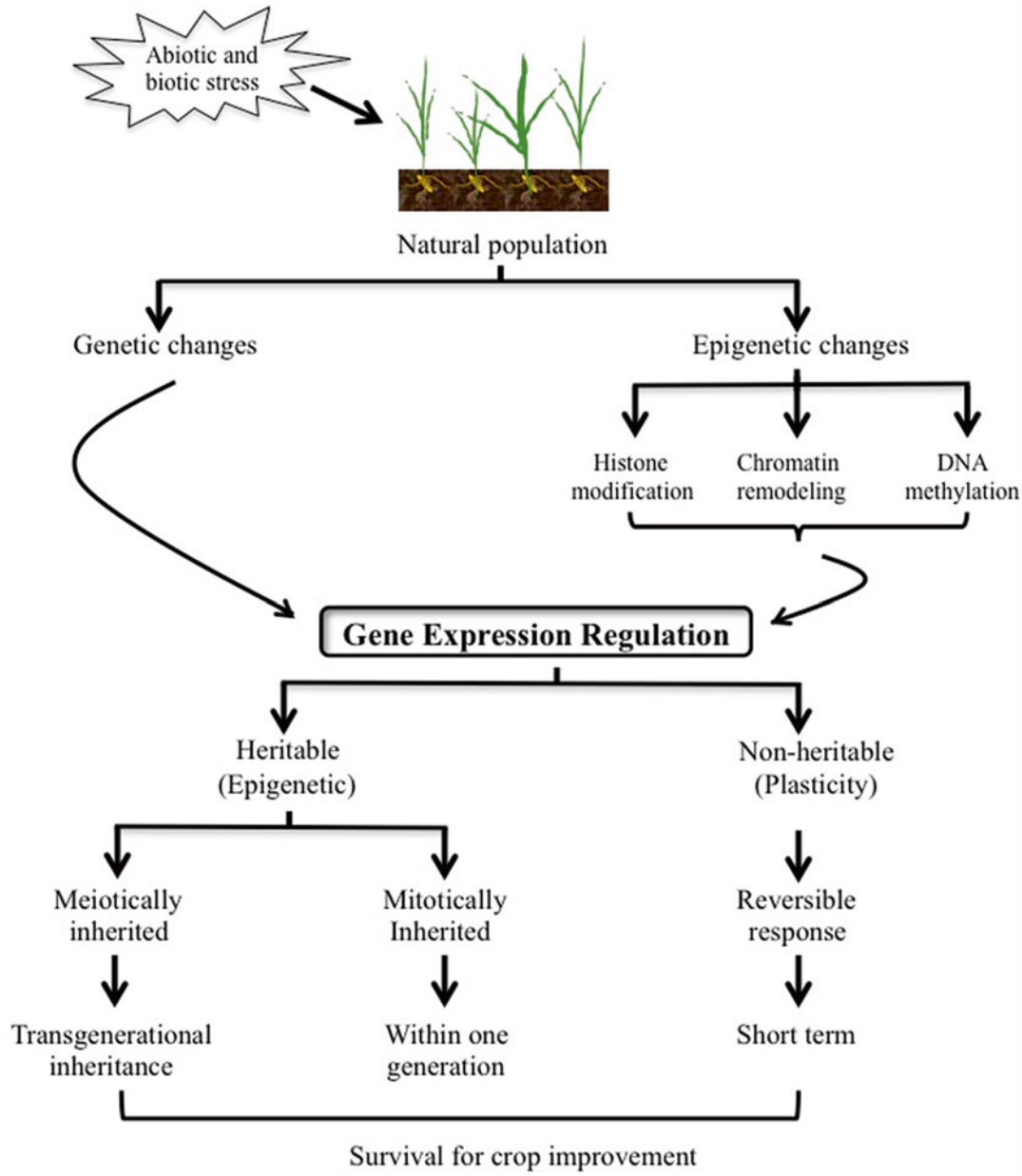

Fig. 1 Gene expression regulation through genetic and epigenetic modifications in natural population in response to environmental stimuli. The genetic and epigenetic changes may act alone or together and regulate the gene expression, which may result in a heritable and nonheritable change and may lead to a survival and/or crop improvement

caused by a genetic variant. The example for this kind of epialleles is DNA methylation spreading into a gene after the insertion of a neighboring transposon where this methylation of the gene is maintained across generations even after the facilitating transposon is excised or segregated away, meaning they could be partly attributable to both genetic and epigenetic differences. The third category is obligate epialleles, which are directly determined by genetic variants and

co-segregate with these methylation variants (Woo et al. 2007). For example, methylation of a gene may be dependent on the presence or absence of a nearby transposon. There are many examples of epimutations that provide evidence that the genetic events like transposon insertions, duplications, and other structural rearrangements might trigger the chromatin remodeling which results in epigenetic control for particular haplotypes (Martin et al. 2009; Durand et al. 2012).

Increasing world population and changing climate demand to improve crop species. Although Mendelian-based genetic approaches and DNA sequence variation to select and improve crop varieties capture a substantial portion of heritable variation, dissecting epigenetic mechanisms could lead to more efficient improvement of crops. A crop improvement strategy includes the response to environmental stimuli, and the potential role of chromatin modifications in biotic and abiotic stresses has been recently reported (Chinnusamy and Zhu 2009; Kim et al. 2010; Berr et al. 2012). In view of global climate change, improving our knowledge of epigenetic regulation could have a significant impact on breeding for increased stress tolerance. In this chapter, we summarize the recent advances in epigenetic regulation in response to stress and discuss the potential of epigenetic regulatory mechanisms for crop improvement.

Histone Modifications

The N-terminal tails of histone are subjected to different covalent posttranslational modifications (PTMs) through the addition of acetyl or methyl groups and small peptide such as ubiquitin. Numerous PTMs may occur on one histone or different histones from the same nucleosome. Histone modifications, particularly acetylation/deacetylation and methylation/demethylation, epigenetically regulate the response to various stresses (Table 1). Here below we summarize the current knowledge of the enzymes responsible for histone modifications and involvement to environmental stimuli.

Histone Acetylation

Histone acetylation is linked to transcriptional activation in euchromatin and also related to DNA replication, recombination, and repair (Allfrey et al. 1964; Allis et al. 1985; Unnikrishnan et al. 2010). Acetylation by addition of an acetyl group to histone lysine (K) residues neutralizes the positive charge of lysine and therefore modifies the histone-DNA interaction, relieving DNA from its condensate state and exposing it to the transcriptional machinery. In *Arabidopsis*, lysine residues of histone H3 (K9, K14, K18, K23, and K27) and H4 (K5, K8, K12, K16, and K20) are subjected to acetylation modifications (Earley et al. 2007; Zhang et al. 2007). Histone acetyltransferases (HATs) are divided into four main classes based on the sequence homology with yeast and mammalian HATs and mode of action: GNAT (GCN5-related *N*-terminal acetyltransferases), MYST (MOZ, Ybt2, Sas2, Tip60 like), CBP/p300 (CREB-binding protein), and TAF1/TAF_{II}250 families (Sterner and Berger 2000). AtGCN5 (GENERAL CONTROL NON-REPRESSIBLE 5) was shown to acetylate H3 in vitro (Earley et al. 2007). *Atgcn5* mutant showed reduced levels of global H3 acetylation (Bertrand et al. 2003), particularly on H3K14 and H3K27 at certain gene loci (Benhamed et al. 2006). AtGCN5 was found to be involved in environmental responses (i.e., cold), along with other development pathways (Vlachonasios et al. 2003). AtGCN5 not only interacts with *Arabidopsis* Ada2 homologues AtADA2a and AtADA2b in vitro but also acetylates AtADA2a/b (Stockinger et al. 2001; Mao et al. 2006). *Atada2b* mutants showed a hypersensitive response to salt and abscisic acid (ABA) and altered response to low-temperature stress (Hark et al. 2009). H3 and H4 acetylation was found reduced on *COR6.6* (*COLD-RESPONSIVE 6.6*), *RAB18* (*RESPONSIVE TO ABA 18*), and *RD29b* (*RESPONSIVE TO DESSICATION 29b*) genes under salt stress in *Atada2b* mutants (Kaldis et al. 2011). The cold-induced transcription factor CBF1 (C-repeat/DRE BINDING FACTOR 1) interacts with AtADA2 and AtGCN5 (Mao et al. 2006), and they positively regulate the

Table 1 Histone modifications and chromatin remodeling factors involved in biotic and abiotic stresses

Type	Gene	Plant	Function	References
HATs				
GNAT	*AtGCN5*	Arabidopsis	Cold stress	Vlachonasios et al. (2003)
	AtABO1	Arabidopsis	Drought and oxidative stress tolerance, ABA sensitive	Chen et al. (2006)
	AtELP2	Arabidopsis	Oxidative stress tolerance, ABA sensitive	Zhou et al. (2009)
	AtELP4	Arabidopsis	ABA sensitive	Zhou et al. (2009)
	AtELP6	Arabidopsis	ABA sensitive	Zhou et al. (2009)
	OsHAG702	Rice	Cold and heat stress, ABA sensitive	Liu et al. (2012)
	OsHAG703	Rice	Cold, drought, and salt stress; ABA sensitive	Liu et al. (2012) and Fang et al. (2014)
	OsHAG704	Rice	Heat stress	Liu et al. (2012)
	HvGCN5	Barley	ABA sensitive	Papaefthimiou et al. (2010)
	HvElp3	Barley	ABA sensitive	Papaefthimiou et al. (2010)
MYST	*OsHAM701*	Rice	Drought and salt stress	Liu et al. (2012) and Fang et al. (2014)
	HvMYST	Barley	ABA sensitive	
CBP/p300	*OsHAC701*	Rice	Cold, heat, and salt stress; ABA sensitive	Liu et al. (2012)
	OsHAC703	Rice	Cold, drought, and salt stress; ABA and SA sensitive	Liu et al. (2012) and Fang et al. (2014)
	OsHAC704	Rice	Cold, heat, and salt stress; SA sensitive	Liu et al. (2012)
TAF1	*OsHAF701*	Rice	Cold and drought stress	Liu et al. (2012) and Fang et al. (2014)
HDACs				
RPD3/HDA1	*AtHDA19*	Arabidopsis	Resistance to *A. brassicicola* and *P. syringae*, salt stress tolerance	Zhou et al. (2005), Chen and Wu (2010), and Choi et al. (2012)
	OsHDA705	Rice	SA, JA, and ABA sensitive	Fu et al. (2007)
	OsHDA714	Rice	Cold, salt, and mannitol stress	Fu et al. (2007)
HD2-like	*AtHD6*	Arabidopsis	Freezing tolerance and JA signaling	
	AtHD2C	Arabidopsis	Salt and drought stress tolerance, ABA sensitive	
	OsHDT701	Rice	Resistance to *M. oryzae* and *Xoo*; SA, JA, and ABA sensitive	Fu et al. (2007), Li et al. (2011), and Ding et al. (2012)
	OsHDT702	Rice	SA, JA, and ABA sensitive	Fu et al. (2007)
	HvHDAC2-1	Barley	SA, JA, and ABA sensitive	Demetriou et al. (2009)
	HvHDAC2-2	Barley	SA, JA, and ABA sensitive	Demetriou et al. (2009)
SIR2	*AtSRT2*	Arabidopsis	Resistance to *P. syringae*, SA signaling	Wang et al. (2010)
	OsSIRT701	Rice	Cold, salt, and mannitol stress	Fu et al. (2007)
	OsSIRT702	Rice	Cold, salt, and mannitol stress	Fu et al. (2007)
	OsSRT1	Rice	Oxidative stress tolerance	Huang et al. (2007)

(continued)

Table 1 (continued)

Type	Gene	Plant	Function	References
HMTs				
Lysine	*AtATX1*	Arabidopsis	Drought stress, SA sensitive	Ding et al. (2011)
	AtSDG8	Arabidopsis	Resistance to *A. brassicicola* and *B. cinerea*, JA/ET	Berr et al. (2010) and Palma et al. (2010)
	HvTX1	Barley	Drought stress	Papaefthimiou and Tsaftaris (2012b)
	HvE(Z)	Barley	ABA sensitive	Kapazoglou et al. (2010)
Arginine	*AtPRMT5*	Arabidopsis	Salt stress tolerance	Zhang et al. (2011)
HDMs				
Jumonji (jmj)	*HvPKDM7*	Barley	Drought stress	Papaefthimiou and Tsaftaris (2012a)
Others				
Ubiquitination	*AtHUB1*	Arabidopsis	Resistance to *B. cinerea* and *A. brassicicola*	Dhawan et al. (2009)
PC Complex	*AtMSI1*	Arabidopsis	Drought stress tolerance	Alexandre et al. (2009)
	HvFIE	Barley	ABA sensitive	Kapazoglou et al. (2010)
Remodelers	*AtCHR12*	Arabidopsis	Drought, heat, and salinity stress	Mlynárová et al. (2007)
	AtBRM	Arabidopsis	Drought stress tolerance	Han et al. (2012)
	AtSYD	Arabidopsis	Resistance to *B. cinerea*	Walley et al. (2008)

Abbreviations: *HAT* histone methyltransferases, *HDAC* histone deacetylases, *HMTs* histone methyltransferases, *HDM* histone demethylases, *PC* polycomb

expression of cold-inducible genes during cold stress (Pavangadkar et al. 2010). This suggests that CBF is recruiting GCN5-containing activator complexes to activate the cold-responsive genes. SGF29 (*SAGA-ASSOCIATED FACTOR 29*), another component of GCN5-containing complexes in yeast, has two orthologs in *Arabidopsis* AtSGF29a and AtSGF29b. *Atsgf29a* mutants showed increased tolerance to salt stress (Kaldis et al. 2011), whereas *Atada2b* mutants were hypersensitive. This suggests that different components of GCN5-containing HAT complexes may play a different role in plant stress tolerance. Elongator HAT complex is involved in ABA signaling, drought, and oxidative stress responses in *Arabidopsis* (Chen et al. 2006; Zhou et al. 2009). *AtABO1/ELO2* (*ABA OVERLAY SENSITIVE 1*), an Elp1 homologue of yeast, was identified in a genetic screen of drought-resistant mutant (Chen et al. 2006). *Atabo1/elo2/elp1* mutant showed ABA hypersensitivity in germination and seedling growth and also showed drought- and oxidative-resistant phenotype (Chen et al. 2006). Mutation in the genes coding for the core subcomplex subunits *AtABO1/ELO2/ELP1* and *AtELP2 (ELONGATOR SUBUNIT 2)*, but not in the genes coding for accessory subcomplex subunits *AtELP4 (ELONGATOR SUBUNIT 4)* and *AtELP6 (ELONGATOR SUBUNIT 6)*, caused stomatal closing to be hypersensitive to ABA (Zhou et al. 2009). Furthermore, these single mutants showed resistance to oxidative stress and to CsCl compared to the wild type plant (Zhou et al. 2009). AtELP2 and AtELP3 *(ELONGATOR SUBUNIT 3)* were also required for both basal immunity and effector-triggered immunity (ETI), but not for systemic acquired resistance (SAR) (DeFraia et al. 2010; DeFraia et al. 2013). These results suggest that elongators play crucial roles in ABA signaling pathways and abiotic and biotic stress responses. AtTAF1/HAF2 was shown to be required for light-regulated gene expression (Benhamed et al. 2006). Together, HATs from GNAT family are involved in both biotic and abiotic stresses. However, involvement of HATs from CBP, MYST, and TAF1 classes in biotic and

abiotic stresses response is still lacking in *Arabidopsis*.

Until now, the knowledge of HATs in the field crops is very limited. Eight HATs have been identified in rice and divided into four families: GNAT (OsHAG702, OsHAG703, and OsHAG704), MYST (OsHAM701), CBP/p300 (OsHAC701, OsHAC703, OsHAC704), and TAF1/TAF_{II}250 (OsHAF701) (Liu et al. 2012). Rice HATs respond to ABA, salicylic acid (SA), and various abiotic stresses, i.e., cold, heat, drought, and salt (Liu et al. 2012; Fang et al. 2014). An increase in transcription of *OsHAG702*, *OsHAG703*, *OsHAC701*, *OsHAC703*, and *OsHAM701* was observed with the exogenous application of ABA, whereas *OsHAC703* and *OsHAC704* transcript levels were reduced with SA application. In addition, *OsHAC701*, *OsHAC703*, *OsHAC704*, and *OsHAG703* transcripts were induced by salt and depressed by cold exposure (Liu et al. 2012). Furthermore, H3 (K9, K18, and K27) and H4 (K5) acetylation and transcripts of *OsHAG703*, *OsHAM701*, *OsHAC703*, and *OsHAF701* were found increased after drought stress in rice seedlings (Fang et al. 2014). Barley HATs belonging to GNAT (HvGCN5 and HvELP3) and MYST (HvMYST) families respond to ABA (Papaefthimiou et al. 2010). The expression of *HvGCN5*, *HvELP3*, and *HvMYST* was induced with exogenous application of ABA (Papaefthimiou et al. 2010). Together, these studies showed that HATs from all the four families are involved in different stresses in field crops. Therefore, the understanding of molecular mechanism may play an important role to cope with various stresses in field crops. It is hoped that this will eventually lead to a long-term improvement of stress tolerance in field crops, which is important for food security.

Histone Deacetylation

The homeostatic balance of histone acetylation is maintained through the antagonistic action between HATs and histone deacetylases (HDACs). In *Arabidopsis*, HDACs are classified into three families: the reduced potassium dependency 3 (RPD3/HDA1) superfamily, the HD2-like family, and the silent information regulator 2 (SIR2) family (Imhof et al. 1997; Sterner and Berger 2000; Strahl and Allis 2000). Functional analysis has demonstrated that HDA1 class of HDACs is involved in both biotic and abiotic stresses response in *Arabidopsis*. Overexpression of *AtHDA19* leads to increased expression of a gene that integrates jasmonic acid (JA) and ethylene (ET) signaling pathway, i.e., *ERF1* (*ETHYLENE RESPONSIVE FACTOR 1*) and *PR* (*PATHOGENESIS RELATED*) genes. This results in increased plant resistance to *Alternaria brassicicola* (Zhou et al. 2005). It is also reported that AtHDA19 (*HISTONE DEACETYLASE 19*) is involved in the repression of SA-mediated defense responses. *Athda19* mutant has increased SA contents and the expression of PR genes, resulting in enhanced resistance to *Pseudomonas syringae* (Choi et al. 2012). AtHDA19 interacts with WRKY38 (*WRKY TRANSCRIPTION FACTOR* 38) and WRKY62 (*WRKY TRANSCRIPTION FACTOR 62*) transcriptional activator to regulate plant basal defense responses (Kim et al. 2008). AtHDA6 (*HISTONE DEACETYLASE 6*), another HDAC, is also involved in JA response, and *Ataxe5/hda6* showed reduced expression of JA-responsive genes *PDF1.2 (PLANT DEFENSIN 1.2)*, *VSP2 (VEGETATIVE STORAGE PROTEIN 2)*, *JIN1 (JASMONATE INSENSITIVE 1)*, and *ERF1* (Wu et al. 2008). *Ataxe5/hda6* mutants also showed reduced freezing tolerance (To et al. 2011), indicating that AtHDA6 has a critical role in freezing tolerance. The expression of ABA and abiotic stress-responsive genes *ABI1 (ABA INSENSITIVE 1)*, *ABI2 (ABA INSENSITIVE 2)*, *KAT1 (POTASSIUM CHANNEL IN ARABIDOPSIS THALIANA 1)*, *KAT2 (POTASSIUM CHANNEL IN ARABIDOPSIS THALIANA 2)*, *DREB2A (DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN 2A)*, *RD29A (RESPONSIVE TO DESSICATION 29A)*, and *RD29B* was decreased in *Ataxe5/hda6* mutant or *AtHDA6-RNAi* plants (Chen et al. 2010). Similarly, *Athda19* mutant also showed a hypersensitive

response to ABA and salt stress (Chen and Wu 2010). This suggests that *AtHDA19* and *AtHDA6* may play a redundant role in modulating ABA and salt stress response. Moreover, AtHDA19 and AtHDA6 play a crucial role in responses to biotic and abiotic stresses. *AtHDA2C*, an HD2-type HDAC, was also shown to be involved in ABA and salt stress response. Overexpression of *AtHD2C* in transgenic plants showed enhanced tolerance to salt and drought stress and ABA-insensitive phenotype (Sridha and Wu 2006). Conversely, *Athd2c* mutant showed a hypersensitive response to ABA and NaCl and decreased tolerance to salt stress (Luo et al. 2012). Furthermore, AtHD2C interacts with AtHDA6 (Luo et al. 2012), suggesting that AtHD2C may functionally associate with AtHDA6 to ABA and salt stress responses and may be a part of HDAC complexes to regulate gene expression through histone modifications. *Arabidopsis SIRTUIN 2* (*AtSRT2*), an SIR2 HDAC expression, is down-regulated upon *Pseudomonas syringae* pv. *tomato* (*Pst*DC 3000) infection. AtSRT2 suppresses the expression of SA biosynthesis genes *PAD4 (PHYTOALEXIN-DEFICIENT 4)*, *EDS5 (ENHANCED DISEASE SUSCEPTIBILITY 5)*, and *SID2 (SPAC24B11.11C)*, thereby supressing SA production and expression of defense-regulated genes (Wang et al. 2010).

HDAC and its involvement in biotic and abiotic stresses have also been reported in cereals. Rice has 19 genes coding for HDAC (Hu et al. 2009), which may play an important role in regulating various stress responses. *OsHDA705*, *OsHDT701*, and *OsHDT702* transcripts were found affected by SA, JA, and ABA, whereas *OsHDA714*, *OsSRT701*, and *OsSRT702* expression is modulated by cold, mannitol, and salt (Fu et al. 2007). In *OsSRT1*-RNAi transgenic rice, H3K9 acetylation and H3K9 dimethylation (H3K9me2) levels were decreased and increased, respectively, leading to H2O2 production, DNA fragmentation, cell death, and lesion-mimicking plant hypersensitive responses during incompatible interactions with pathogens. In contrast, *OsSRT1* overexpression showed an enhanced tolerance to oxidative stress (Huang et al. 2007). Overexpression of *OsHDT701*, a plant-specific HD2 HDAC, leads to decreased level of H4 acetylation on flowering and defense-related genes and enhanced susceptibility to the *Magnaporthe oryzae* and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) pathogens (Li et al. 2011; Ding et al. 2012). In contrast, silencing of *OsHDT701* showed increased levels of H4 acetylation and increased transcription of pattern recognition receptor (PRR) and defense-related genes, elevated generation of reactive oxygen species, as well as enhanced resistance to both *M. oryzae* and *Xoo* (Ding et al. 2012). *HvHDAC2-1* and *HvHDAC2-2* genes, HD2-type HDAC from barley, were found to respond to JA, ABA, and SA treatments, implying an association of these barley genes with plant resistance to biotic and abiotic stresses (Demetriou et al. 2009). *NtHD2a* and *NtHD2b* genes, HD2-type HDAC from tobacco, were found to work as inhibitors of cryptogein-induced cell death (Bourque et al. 2011). Together, HD2-type HDAC from rice and barley carries the same function, suggesting a common function among species for HDAC homologues but also possible species-specific functional diversification, in response to stress. The involvement of HDACs in biotic and abiotic stresses response in agronomically important crops and their underlying molecular mechanism is of utmost importance for sustainable crop improvement.

Histone Methylation

Histone methylation plays an essential role in diverse biological processes ranging from transcriptional regulation to heterochromatin formation. Methylation of histone can occur on lysine (K) or arginine (R) residues leading to either transcriptional activation or repression. Histone methylation not only occurs at different residues (K and R) and distinct sites (e.g., K4, K9, K27, K36, R2, and R17 of H3 and K20 and R3 of H4, etc.) but also differs in the number of methyl groups added (mono-, di-, and tri-methylated). Methylation of lysine residues does not affect their net charge but elevates the hydrophobicity nature of the side chain and may alter intra-

or intermolecular interactions or create new binding surfaces for proteins that bind preferentially to the methylated domains (Liu et al. 2010). Indeed, the arginine residue, after the addition of the methyl group, changes its shape and removes a potential hydrogen bond donor as well (Bedford and Clarke 2009). Mostly the studies have been done on histone modifications only at individual stress-induced plant genes. Very few studies with genome-wide histone methylation analysis have been reported. van Dijk et al. (2010) have studied genome-wide analysis of the histone H3 lysine 4 mono-, di-, and tri-methylation (H3K4me1, H3K4me2, H3K4me3, respectively) patterns in chromatin isolated from *Arabidopsis* rosette leaves before and after dehydration stress. Genome-wide transcript patterns in watered and dehydration-stressed plants were compared in this study. The presence of the H3K4me1, H3K4me2, and H3K4me3 marks is predominantly located on genes, and the distribution of H3K4me1 and H3K4me2 is higher than H3K4me3. Interestingly, H3K4me1, H3K4me2, and H3K4me3 patterns display different dynamics and specific patterns at upregulated, downregulated, and unaffected genes during the response to dehydration stress. A modest change in H3K4me2 and H3K4me1 levels was found at a subset of known stress response genes, but the H3K4me3 abundance over gene bodies changed more dramatically at genes whose transcript levels increased or decreased during dehydration. The different behaviors of each methylation mark during the response process illustrate that each mark plays a distinct role in the transcriptional response of implicated genes. In a recent study, genome-wide profiling of histone H3K4-trimethylation of 25-day-old rice plants under dehydration conditions was done. This analysis uncovered a positive correlation between H3K4me3 accumulation and the expression levels of some drought-responsive genes during dehydration. This correlation could be extended to genes involved in stress-related metabolite and hormone signaling pathways (Zong et al. 2012). These genome-wide histone modification studies help broaden our knowledge on whole genome scale and indicate a need to study histone modifications on a genome-wide level in response to other abiotic stresses as well.

Histone Lysine Methylation

Covalent addition of one, two, or three methyl groups (me1, me2, or me3) mainly occurs on H3K4, H3K9, H3K27, H3K36, and H4K20, and this function is exerted through histone methyltransferases (HMTs). All known plant HMTs have a so-called SET [from the initially identified *Drosophila* HMTs: Suppressor of variegation (Su(var)3-9), Enhancer of Zeste (E(z)), and Trithorax (TRX)] catalytic domain, an evolutionarily conserved sequence of 130–150 amino acids in length. SET Domain Group (SDG) proteins are classified into three subgroups: Su(var)3-9, Enhancer of Zeste (E(z)), and Trithorax (TRX). These subgroups have been shown to establish different chromatin marks, leading to different impacts on transcription. SDGs of the ASH1 and TRX subgroups primarily belong to the Trithorax group (TrxG) and are responsible for methylation on H3K36 and/or H3K4, which are associated with transcriptional activation (Agger et al. 2008; Liu et al. 2010). The E(z) subgroup SDGs catalyze H3K27 methylation associated with transcriptional gene silencing. H3K27 can be mono-, di-, and trimethylated and seems to be one of the major gene silencing mechanisms in *Arabidopsis* because ~17 % of the coding genes were marked with H3K27me3 (Turck et al. 2007). Classically and conservatively, the Su(var)3-9 subgroup SDGs potentially show an H3K9 methyltransferase activity and are associated with inactive genes located in a euchromatic region and within highly condensed constitutive heterochromatin (Ng et al. 2007). SDG proteins have been involved in diverse biological processes, including flowering time regulation, floral organogenesis, leaf morphogenesis, parental imprinting, and seed development (Liu et al. 2010; Berr et al. 2011; Shafiq et al. 2014). AtATX1/SDG27, a member of the Trithorax group, is a methyltransferase of H3K4me3. AtATX1 was found to be involved in drought and SA pathway responses (Ding et al. 2011; Berr et al. 2012). *Atatx1* mutant displayed larger stomatal apertures, increased transpiration,

and decreased tolerance to dehydration stress. AtATX1 is required for the induction of *NECD*, a gene involved in ABA biosynthesis and deposition of H3K4me3 in response to dehydration stress. AtATX1 can influence gene expression by ABA-dependent as well as ABA-independent pathways (Ding et al. 2011). AtATX1 was described as critical for basal resistance against *Pst* DC3000, and it regulates the SA-inducible expression of transcriptional factor *WRKY70* (Alvarez-Venegas et al. 2006; Berr et al. 2012). AtSDG8, another member of the Trithorax group, is the major H3K36me2/me3 methyltransferase (Xu et al. 2008). AtSDG8 was reported in *Pst* DC3000-triggered plant defense through the regulation of particular *R* genes (Palma et al. 2010) and the transcriptional activation of JA/ET signaling-related genes (Berr et al. 2010). *Atsdg8* mutant exhibited reduced resistance to *Alternaria brassicicola* and *Botrytis cinerea* (Berr et al. 2010). H3K36 methylation on defense-related genes is impaired in *Atsdg8* mutant (Berr et al. 2010; Palma et al. 2010), indicating that AtSDG8 mediates the pathogen response by regulating histone methylation of defense-responsive genes. The expression of *AtSUVH2*, *AtSUVH5*, *AtSUVH6*, and *AtSUVH8* genes encoding H3K9 methylation decreased in the progenies of salt-stressed plants (Bilichak et al. 2012). In addition, *Curly leaf* (*CLF*) gene encoding H3K27 methylation was hypermethylated in the progenies of salt-stressed plants (Bilichak et al. 2012). These results suggest that H3K9 and H3K27 methyltransferases are involved in the plant stress adaptation. Until now, HMT involvement in biotic and abiotic stresses response is very limited in crops. HvTX1, barley TRX-like H3K4 methyltransferase, has been shown to be involved in drought stress. The transcripts of *HvTX1* were found increased under drought stress (Shvarts Iu et al. 2010; Papaefthimiou and Tsaftaris 2012b). This suggests that TrxG plays an important role in plant response to environmental stresses. A homologue of polycomb complex subunit from barley HvE(Z) was found to be induced by ABA implying an association with ABA-mediated processes during seed development and stress response (Kapazoglou et al. 2010). Recently, it was shown that 18 genes containing SET domain from maize showed differential expression under salt and drought stress (Qian et al. 2014). Although SET domain proteins are involved in biotic and abiotic stresses in crops, their molecular mechanism is still missing. It is hoped that with emerging new technologies and better understanding of molecular mechanism, the SET domain proteins may have potential for sustainable crop improvement.

Histone Arginine Methylation

Arginine methylation mainly occurs at R2, R8, R17, and R26 of histone H3 and R3 of histone H4 and histone H2A. Arginine methylation can be symmetric or asymmetric and only occurs in mono- and di-methyl states (Aletta et al. 1998; Bedford and Clarke 2009). Arginine methylation is evolutionarily conserved and has been found in fungi, plants, *Caenorhabditis elegans*, *Drosophila melanogaster*, and vertebrates (Krause et al. 2007). Arginine methylation is catalyzed by a small group of protein arginine methyltransferases (PRMTs) that harbor a set of four conserved motifs (i.e., I, post-I, II, III) and a THW loop (Katz et al. 2003). Proteins that are arginine methylated play an essential role in transcriptional regulation, DNA repair, signal transduction, nuclear/cytoplasmic shuttling, RNA processing, and formation of silent chromatin (Bedford and Richard 2005; Bedford and Clarke 2009). In mammals, PRMTs are classified into two classes depending on the nature of the modification introduced. Although both type I and type II catalyze arginine monomethylation, they differ in the final type of arginine modification. The type I enzymes result in asymmetrical dimethylarginine, whereas type II enzymes result in symmetrical dimethylarginine (McBride and Silver 2001; Katz et al. 2003; Jelinic et al. 2006). The involvement of arginine methylation in biotic and abiotic stresses is very poorly understood. AtPRMT5/SKB1, a homologue of the human PRMT5 (*PROTEIN ARGININE METHYLTRANSFERASE 5*), specifically dimethylates symmetrically H4R3 as a type II arginine methyltransferase in *Arabidopsis* (Deng et al. 2010). *Atskb1* mutant displayed salt-

hypersensitive phenotype. AtSKB1 suppresses the transcription of stress-responsive genes by increasing the H3R3sm2 (Zhang et al. 2011).

Histone Demethylation

Histone methylation is important for chromatin stability and gene expression and was considered irreversible until the discoveries of demethylases that antagonized or balanced the methylase activities. There are two types of methylases with distinct mechanisms, the lysine-specific demethylases (LSD1) and the Jumonji C (JmjC) domain-containing demethylases. They use different cofactors and act on different substrates to remove methyl groups from methylated lysine residues. LSD1 is catalytically limited to mono- and di-methylated lysine due to the reaction mechanism used to initiate the demethylation (Klose and Zhang 2007). Unlike LSD1, Jmj proteins do not have limitations in their catalytic mechanism and are able to demethylate mono-, di-, and tri-methyl residues (Agger et al. 2008). In *Arabidopsis* and rice, histone demethylases (HDMs) have been found to be involved in many developmental processes and gene silencing (Noh et al. 2004; Sun and Zhou 2008; Chen et al. 2013; Cui et al. 2013; Shafiq et al. 2014). Although the role of HDMs in stress response is not yet clear, evidences suggest that histone demethylation may be involved in stress responses. Increased level of H3K9/K14ac and H3K4me3 and decreased level of H3K9me2 on ABA-responsive genes (*ABI1*, *ABI2*, and *RD29B)* have been found in *Arabidopsis* after ABA treatment (Chen et al. 2010), which suggests that some HDMs are working for the demethylation of H3K9 to activate the ABA-responsive genes. Decreased levels of H3K4me1, H3K4me2, and H3K4me3 and downregulation of stress-responsive genes have been reported upon dehydration stress in *Arabidopsis* (van Dijk et al. 2010). This also suggests that HDMs are modulating the expression of stress-responsive genes. Recent reports describing a putative role of HDMs in stress response are anticipated. Putative plant-specific barley HvPKDM7 histone demethylase was found to be significantly induced by drought stress (Papaefthimiou and Tsaftaris 2012a). Genome-wide analysis of rice showed that a lot of genes were differentially H3K4me3-modified in drought stress (Zong et al. 2012), suggesting that the rice HDMs are involved in stress response.

Other Histone Modifications

Ubiquitination is the covalent attachment of a small (76 amino acids) and highly conserved protein named ubiquitin to the target protein, achieved through the sequential action of the ubiquitin-activating enzyme E1, the ubiquitin-conjugating enzyme E2 (Ubc), and the ubiquitin-protein ligase E3 (Pickart 2001; Smalle and Vierstra 2004). The substrate can remain monoubiquitinated, or the ubiquitin can have several lysine (K) residues that may be substrates themselves for subsequent addition of ubiquitins, resulting in a polyubiquitin chain. H2B monoubiquitination (H2Bub1) in yeast, animals, and *Arabidopsis* is mainly associated with transcriptional activation (Briggs et al. 2002; Dover et al. 2002; Hwang et al. 2003). AtHUB1, catalyzing H2B monoubiquitination, was reported as a regulatory component of plant defense against necrotrophic fungal pathogens (Dhawan et al. 2009). *Athub1* mutant displayed susceptibility to *B. cinerea* and *A. brassicicola*. ET and SA but not JA modulate the resistance of *Athub1* mutants to necrotrophic fungi. *Athub1-6* presents a reduced cell thickness, indicating that HUB1 may regulate resistance by altering plant cell wall-related defense mechanisms (Dhawan et al. 2009). It remains to be explored whether and how H2Bub is involved in plant defense. AtMSI1 (MULTICOPY SUPPRESSOR OF IRAI), a subunit of the Polycomb group (PcG) having H3K27 methylation activity, has been shown to be involved in drought stress (Alexandre et al. 2009). *Atmsi1* mutant displayed increased tolerance to drought stress and increased transcripts of stress and ABA-responsive genes, indicating that AtMSI1 suppresses stress-responsive genes in an ABA-dependent manner. Polycomb complex gene homologue from barley HvFIE

(*FERTILIZATION-INDEPENDENT ENDOSPERM 1*) was found to be induced by ABA implying an association with ABA-mediated processes during seed development and stress response (Kapazoglou et al. 2010).

ATP-Dependent Chromatin Remodeling Factors

ATP-dependent chromatin remodeling complexes use the energy of ATP hydrolysis to alter the structure of chromatin for the regulation of gene expression (Vignali et al. 2000). ATP-dependent chromatin remodeling factors have been found to be involved in biotic and abiotic stresses response. ATP-dependent chromatin complexes can be grouped into three classes: the SWI/SNF ATPases, the imitation switch (ISWI) ATPases, and the chromodomain and helicase-like domain (CHD) ATPases. AtCHR12, an SNF/BRAHMA-type (BRM) chromatin remodeling factor, was shown to be involved in plant growth response to adverse environmental conditions (Mlynárová et al. 2007). Under drought, heat, and salinity stress, *AtCHR12* overexpressing plants exhibited an arrested growth of normally active primary buds as well as reduced growth of primary stem. *Atchr12* mutant plants displayed less growth arrest than the wild type when exposed to stress. However, the molecular mechanism of how the AtCHR12 is involved in growth arrest under adverse environments is not clear. AtBRM, an SWI2/SNF2 chromatin remodeling ATPase, has been demonstrated to be involved in drought stress (Han et al. 2012). *Atbrm* mutant showed increased drought tolerance and regulated the expression of ABA-responsive gene *AB15*. Furthermore, nucleosomes were found destabilized upon the loss of BRM activity, indicating that BRM regulates stress responses through the regulation of nucleosome stability of *AB15*. Using wounding as stimulus, SPLAYED (SYD), a closest homologue of BRM, was shown to be required for the basal as well as stress-induced expression of genes (*PDF1.2*, *VSP2*, and *MYC2*) working downstream of JA/ET signaling pathways (Walley et al. 2008). These results indicate that ATP-dependent chromatin remodeling complexes are playing a crucial role in stress responses, thus influencing plant innate immunity and tolerance. However, the knowledge about the ATP-dependent chromatin remodeling factors in field crops is very limited. After UVB treatment of maize plants, the enrichment of SWI2/SNF2 was found at target genes, implying the involvement of chromatin remodeling factors in abiotic stress responses (Casati et al. 2008). It is expected that by exploiting the rice, maize, and *Brachypodium* genomes, chromatin remodeling complexes and their association with biotic and abiotic stresses will be studied, thus improving crop production.

DNA Methylation

DNA methylation refers to a chemical modification of genomic DNA by the addition/attachment of a methyl (–CH3) group to specific nucleotide bases, which could be cytosine or adenine. It occurs most commonly on cytosine base leading to a 5-methylcytosine. It is conserved in major eukaryotic groups, i.e., plants, animals, and fungi, with few exceptions (Goll and Bestor 2005; Henderson and Jacobsen 2007). Although methylation at cytosine can be explained in a variety of DNA sequence contexts, mechanistically it can be classified broadly into three contexts, CG, CHG, and CHH (where H denotes A, T, or C) (Law and Jacobsen 2010). The pattern of occurrence of DNA methylation varies, i.e., it mainly occurs at CG sites in mammals, but it can occur in CG, CHG, and CHH contexts in plants (Feng et al. 2010). In *Arabidopsis*, the genome-wide DNA methylation level is reported to be 24 %, 6.7 %, and 1.7 % for CG, CHG, and CHH contexts, respectively. Alteration in DNA methylation is associated with gene regulation and transposable element silencing in eukaryotes (Law and Jacobsen 2010). It acts differently in different regions of the genome. In transposable elements (TE), where it appears in all three contexts (CG, CHG, and CHH), it is responsible for transcriptional silencing. In genes, DNA methylation is mainly restricted to CG sites (Law and

Jacobsen 2010; Zhang et al. 2010), although CHG and CHH methylation has also been reported recently (González et al. 2011). The presence of DNA methylation at the gene promoter region is generally negatively correlated with gene expression (Zhang et al. 2006; Li et al. 2012). Furthermore, DNA methylation can also occur within the gene body (i.e., away from the 5′ and 3′ ends of transcription units), in the so-called bell-shaped CG "gene body methylation" pattern. However, the function of gene body methylation is still not clear (Zhang et al. 2010).

DNA METHYLTRANSFERASE 1 (MET1) primarily maintains CG methylation, which is a homologue of the mammalian DNA METHYLTRANSFERASE1 (DNMT1). Moreover, CHROMOMETHYLASE 3 (CMT3) maintains CHG methylation. Maintenance of CHH methylation is complex because it is asymmetrical; it needs to be reacquired *de novo* after each replication, through the action of the plant-specific RNA-dependent DNA methylation (RdDM) pathway (Law and Jacobsen 2010) in which small RNAs (24 nucleotides long) target the *de novo* methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) to homologous genomic loci to establish DNA methylation. The modifications in DNA methylation in response to environmental stress have been reported in both locus-specific and genome-wide studies (Table 2). Some of such examples are explained below: In tomato, altered DNA methylation levels were observed on *Asr1* (*ABSCISIC ACID STRESS RIPENING 1*) gene under drought stress (González et al. 2011). *Asr1* is a non-transposon, protein-coding, water stress-inducible gene of the LEA superfamily in tomato. The expression of *Asr1* increases with the longer duration of drought stress. In addition, the CHH methylation was decreased in drought conditions showing the negative correlation with gene expression (González et al. 2011). Furthermore, the existence of all the three contexts of DNA methylation (CG, CHG, CHH) was reported in the regulatory region of *Asr2* (*ABSCISIC ACID STRESS RIPENING 2*). Interestingly, the gene body methylation was restricted to only one context (CG). The site-specific removal of methyl marks from CNN sites in the regulatory region was observed under drought stress. This response of *Asr2* is heritable through generations and could have evolutionary importance (González et al. 2013). In maize, genome-wide DNA methylation pattern was studied under cold stress. It led to the identification of a fragment named *ZmMI1*, which was transcribed only in cold stress conditions (Steward et al. 2002). About 49 transcription factors showed differential expression in soybean on exposure to salinity stress. Moreover, DNA methylation and expression profiles of one *MYB (MYELOBLASTOSIS)*, one *b-ZIP (BASIC LEUCINE ZIPPER)*, and two *AP2/DREB* transcription factor gene family members were significantly correlated (Song et al. 2012). Choi and Sano (2007) analyzed glycerophosphodiesterase-like protein (*NtGPDL*) gene in tobacco to understand the effect of various stresses, including aluminum, salt, and cold stress. The increased transcription and CG demethylation in the coding regions of *NtGPDL* were observed under stress conditions (Choi and Sano 2007). Change in DNA methylation pattern in response to biotic stress was also reported. In tobacco, a pathogen-responsive gene *NtAlix1 (ALG-2 INTERACTING PROTEIN X 1)* was studied upon the infection of tobacco mosaic virus (TMV). The change of DNA methylation at *NtAlix1* was observed after 24 hours of infection, indicating that DNA methylation pattern undergoes alteration in response to biotic stresses which is closely related to the activation of stress-responsive genes (Wada et al. 2004). One interesting example came from the analysis of *Arabidopsis*, where a putative small RNA target region about 2.6 kb upstream of the ATG start codon of *AtHKT1* (*HIGH-AFFINITY K + TRANSPORTER 1*) gene is normally heavily methylated and its hypomethylation represses the *AtHKT1* gene expression, which is crucial for salt tolerance (Baek et al. 2011). In *Arabidopsis*, the promoter region of *AtRMG1 (RESISTANCE METHYLATED GENE 1)* gene is targeted by RdDM and ROS1-dependent DNA demethylation as a defense response against the *P. syringae* pathogen (Yu et al. 2013). Sharma et al. (2009) character-

Table 2 DNA methylation modifications involved in biotic and abiotic stresses

Sr. No.	Genomic region	Plant species	Stress	Mode of action	References
1	*Asr1*	Tomato	Drought stress	CG hypermethylation and CHH hypomethylation	González et al. (2011)
2	*Asr2*	Tomato	Drought stress	CHH hypomethylation in regulatory region	González et al. (2013)
3	*NtGPDL*	Tobacco	Aluminum, salt, and cold	Hypomethylation	Choi and Sano (2007)
4	*NtAlix1*	Tobacco	Tobacco mosaic virus	Alteration in DNA methylation pattern	Wada et al. (2004)
5	*ZmMI1*	Maize	Cold stress	Root-specific hypomethylation	Steward et al. (2002)
6	*AtHKT1*	Arabidopsis	Salt tolerance	Loss in cytosine methylation in a putative small RNA target region	Baek et al. (2011)
7	*RMG1* promoter	Arabidopsis	*P. syringae*	RMG1 is targeted by RdDM and ROS1-dependent DNA demethylation	Yu et al. (2013)
8	*Glyma11g02400* (Promoter)	Soybean	Salinity stress	−518 to −274 cytosines were demethylated following exposure to salinity stress for 1–24 h	Song et al. (2012)
9	*Glyma16g27950 (Promoter)*	Soybean	Salinity stress	Hypomethylation at transcription start codon (24 to 233)	Song et al. (2012)
10	*Glyma20g30840 (Promoter)*	Soybean	Salinity stress	Hypomethylated cytosines at promoter region 1 (−87 to 163)	Song et al. (2012)
11	Genome-wide	Maize	Cold stress	Global methylation shift. Mainly demethylation of fully methylated fragments	Shan et al. (2013)
12	Genome-wide	Mangrove	Salinity stress	Global hypomethylation	Lira-Medeiros et al. (2010)
13	Genome-wide	Rice	Salinity stress	Differential methylation of salt stress-related genes, retrotransposons, and chromatin modifier genes	Karan et al. (2012)
14	Genome-wide	Rice	Drought stress	Genotype-dependent differential methylation	Wang et al. (2011)
15	Nuclear genome	*Mesembryanthemum crystallinum*	High salinity	CHG hypermethylation	Dyachenko et al. (2006)

ized ten putative DNA methyltransferases in rice. Expression analysis of them was done at different developmental stages and under abiotic stress. High salinity and cold stress induced *OsCMT2*, but drought stress showed no effect. Drought and salinity stress caused *OsCMT3* to exhibit approximately a six- and four fold reduction in mRNA accumulation in rice seedlings subjected to high-salt and dehydration conditions, respectively. In addition to locus-specific stress responses, a good deal of work has been done on the genome-wide level. Genome-wide DNA methylation response to cold stress by MSAP (methylation-sensitive amplification polymorphism) technique in maize revealed global DNA methylation shift. The main part of this shift was attributed to the demethylation of fully methylated fragments (Shan et al. 2013). Lira-Medeiros et al. (2010), in an interesting comparative study of mangrove plants, growing in salt marsh neighborhood and riverside habitat, revealed that riverside plants were much taller and thicker than the plants growing in salt marsh neighborhood. Genome-wide DNA methylation analysis showed considerable hypermethylation in riverside plants in comparison with the plants growing in salt marsh neighborhood suggesting a pivotal role of natural epigenetic variations in a plant population toward environmental adaptation. Similarly, a genome-wide study by MSAP analysis performed in diverse rice genotypes differing in their salt-responsive characteristics highlighted differential methylation and expression of salt stress-related genes, retrotransposons, and chromatin modifier genes (Karan et al. 2012). Another study of genome-wide DNA methylation analysis under drought stress has been reported in rice. In this study, the comparison of two genotypes under drought stress and subsequent recovery revealed the genotype-specific DNA methylation modifications, which were mostly reversed after recovery, but some were maintained even after recovery indicating some sort of stress memory. This study illustrated the importance of these induced epigenetic changes in regulatory mechanisms for adaptation of rice plant to environmental stresses (Wang et al. 2011). Dyachenko et al. (2006) reported hypermethylation of CHG methylation in nuclear genome of *Mesembryanthemum crystallinum* plants during high-salinity stress imposition. These examples provide a glimpse of the importance of epigenetic mechanisms in the plant response to the environmental variation and their potential involvement in the adaptive strategies devised by the plants. In this respect, the reported data sets of various plant methylomes could provide the basis for the selection of differential epigenetic regions as probable targets for the genetic manipulation for crop improvement.

Epigenetic Outlook for Crop Improvement

One very significant part of the success attained in the field of crop yield improvement is attributed to the plant breeding and genetics. The utilization of desirable available variation has been one of the main roles followed by the scientists for the improvement of crop plants. In the last two decades, the researchers across the globe have accumulated the wealth of knowledge that provides the evidence of prevalence of epigenetic variability (natural as well as generated) and its potential to influence the phenotype (agronomic traits) and large crop improvement. Histone modifications are involved in mitotically stable transcriptional activation or repression and exhibit lower level of transgenerational heritability. So, can the mitotically stable epigenetic information be used for crop improvement? The answer is that very similar to transcriptional factors, chromatin changes also control plant morphology and response to the environment, and a greater control over traits may be achieved by understanding these mechanisms, which is highly important for the breeding point of view. Several cases of naturally occurring epialleles (i.e., DNA methylation alleles that are independent of DNA sequence variation causing a visible phenotype) have been described, such as the L*cyc* locus in *Linaria vulgaris* (Cubas et al. 1999) and an SBP-box gene in tomato (Manning et al. 2006). DNA methylation of natural epialleles has also been described at a larger genomic scale for species such as

Arabidopsis (Cervera et al. 2002; Vaughn et al. 2007), *Spartina anglica* (Salmon et al. 2005), or *Populus trichocarpa* (Raj et al. 2011). These examples indicate the existence of epigenetic variations in natural populations. Therefore, epigenetic variants could be used in breeding programs for the improvement of crops because breeders select for a particular trait rather than a molecular mechanism.

These genome-specific techniques have important implications on the crop improvement by their potential role in the identification of regions, which show epigenetic modifications under various kinds of stress. This identification could lead to the characterization of these regions of interests in the genome. Further studies of these regions could lead to detection of epialleles which could be incorporated into the breeding programs and play their role in crop improvement. Moreover, recent techniques enabled breeders to generate desired allelic variation through the mutagenesis or transgenic modifications to develop a trait not observed in natural population. Epigenetic regulation affects transgene behavior and could be used to establish novel epialleles for breeding purposes. Different approaches have been proposed, speculated, and/or initiated to use the epigenetic diversity in the breeding programs. One big hurdle in producing or developing epigenetic diversity in crop plants is the lack of availability of genome-wide DNA methylation mutants like *met1* and *ddm1*. In such cases, the usage of chemical inhibitors like 5-azadeoxycytidine is a good alternative. Different studies have reported the transgenerational inheritance of the modifications created by its treatment. (Sano et al. 1990; Akimoto et al. 2007). Akimoto et al. (2007) reported that in progenies of 5-azadeoxycytidine-treated rice, some of the altered phenotypes were stably inherited even after 10 years. Some of these phenotypes were of interest from the breeding point of view like resistance of a bacterial pathogen *Xanthomonas oryzae.* Another interesting and exciting approach, which is drawing much attention, is the usage of epigenetic- Recombinant Inbred Lines (epiRILs). These lines are created by artificial crossing of DNA methylation mutants, i.e., *decreased DNA methylation 1* (*ddm1*) or *methyltransferase 1* (*met1*) with their wild types (Johannes et al. 2009; Reinders et al. 2009). Since these mutants are deficient in DNA methylation machinery but genetically similar as that of the wild type, the resulting lines (epiRILs) have almost identical DNA sequences but divergent patterns of DNA methylations. These patterns are reported to be stable across many generations through molecular analysis. Analysis of these lines has shown that they have widespread phenotypic variation for morphological or developmental traits, like flowering time, plant height, as well as biotic and abiotic stresses (Johannes et al. 2009; Reinders et al. 2009; Zhang et al. 2013). Although these methylation variants do not necessarily reveal natural variation, they can be very useful in multiple ways. They can serve as a good material to understand the extent and potential role of the epigenetic variations, which are independent of genetic variations. They can also help to understand the extent of the phenotypic variation caused by the random combination of plant-specific epigenome. The above mentioned hypothesis was confirmed from the various recent publications giving further insight into the basic mechanisms which require DNA methylation, like the effect of DNA methylation on crossing over where the results showed that the distribution of crossing over event is sensitive to DNA methylation, but the rate of crossing over is not affected by it (Colomé-Tatché et al. 2012; Mirouze et al. 2012). Similarly, significant heritable variation in growth rate in response to biotic stresses was reported. All these results further support the opinion that considerable heritable variations in economically important traits could be created by variation in the DNA methylation patterns, and these kinds of approaches could be applicable for crop improvement. Utilization of epialleles generated and/or identified by various researchers in diverse plant/crop species should be exploited in different breeding programs. Such kind of program could start with the identification and understanding of epigenetic pattern in individuals of the selected population. This will lead to the identification of

specific phenotypes and, finally, the association studies of that inherited phenotype and epigenetic variation. With the advances in the genome editing technologies, the usage of locus-specific epigenetic modifications could also be used for crop improvement (Chen and Gao 2014).

Techniques Used in Epigenetic/ Epigenomic

There are different techniques in use for the detection of DNA methylation profile and DNA-protein interaction both at locus-specific level and on genome-wide level. Some examples of these techniques are discussed here. On locus-specific level, the DNA methylation profile can be studied through bisulfite treatment technique and through the use of methyl-sensitive restriction enzymes. On a genome-wide level, DNA methylation profile can be studied through MSAP (methyl-sensitive amplification polymorphism) (Yaish et al. 2014), through the use of HPLC (high-performance liquid chromatography) technique (Friso et al. 2002). A short description of these techniques is given below.

DNA Methylation

Bisulfite Treatment

In bisulfite treatment, the genomic DNA is treated with sodium bisulfite, which converts all the non-methylated cytosines into uracil. This conversion is followed by the PCR through specific primers. All the uracil (non-methylated cytosines before bisulfite treatment) and thymine residues (which were always thymines even before bisulfite treatment) are being amplified by PCR as thymine, whereas only 5-methylcytosine residues are amplified as cytosine. After sequencing of PCR product, the analysis of sequences provides the information about the methylated sites in the amplified region as well as the methylation level in a particular genomic region (Frommer et al. 1992). With the advancement in the sequencing technology, this technique can also be used in genome-wide methylation analysis.

Methylation-Sensitive PCR (MSP)

Methylation-sensitive PCR (MSP) is a modification in the above-described bisulfite treatment technique where the amplification is done with primer pair that is specific for methylated DNA and primer pair specific to unmethylated DNA (Herman et al. 1996). This technique can be used in the development of epigenetic markers to be used in marker-assisted selection.

Methyl-Sensitive Restriction Enzyme Technique

In methyl-sensitive restriction enzyme technique, the genomic DNA is digested with the methylation-sensitive restriction enzyme, and this digested DNA is amplified by primers flanking the restriction site. PCR will work only if the restriction site is not cleaved (due to the methylation at that site) (Singer-Sam et al. 1990). This kind of technique along with its various modified forms paves the way for the development of simple and practical epigenetic marker for the epialleles which could have implications in marker-assisted selection and could have important role in the crop improvement strategies.

Methyl-Sensitive Amplification Polymorphism (MSAP)

Methyl-sensitive amplification polymorphism (MSAP) is a technique for the DNA methylation analysis on a genome-wide level, in which digestion of genomic DNA is done with a methylation-sensitive restriction enzyme like *Hpa*II as a first step. This is followed by the ligation of DNA fragments to adaptors, which facilitate the amplification of these fragments. *Msp*I, a methylation-insensitive isoschizomer of HpaII, is used in parallel for digestion, and this digestion serves as a loading control in the experiment. After that, amplification of these fragments through fluorescently labeled primers is done. Comparison of PCR products from different individuals allows the user to identify the interesting fragments. This leads to the isolation and characterization of that fragment (Yaish et al. 2014).

High-Performance Liquid Chromatography Technique (HPLC)

In high-performance liquid chromatography technique (HPLC), the genomic DNA is enzymatically hydrolyzed, and this hydrolyzed DNA is then separated into its four major DNA bases and 5-methyl-2′-deoxycytidine using HPLC. 5-Methyl-2′-deoxycytidine is obtained. The global DNA methylation status is calculated by comparing the amount of 5-methyl-2′-deoxycytidine per microgram of DNA with percent relative standard deviations (%RSD) (Friso et al. 2002).

Methylated DNA Immunoprecipitation

It is another technique to study genome-wide changes in DNA methylation patterns. In this technique, DNA is isolated from cells and sheared through sonication. By the usage of antibodies specifically targeting methylated DNA fragments, isolation of methylated regions occurs, which then can be identified using high-resolution DNA microarrays or next-generation sequencing techniques. The global changes in the methylation patterns across the varied cells can be detected through this technique.

Chromatin Immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) is a technique used to study the interaction between proteins (e.g., histones) and DNA. The use of highly specific antibodies directed against DNA-binding proteins is required in this technique, and it can be followed by various nucleic acid analysis techniques, including PCR, qPCR, sequencing, and microarray hybridization. It can help to determine whether certain proteins are associated with specific genomic regions and is also useful for identifying regions of the genome associated with specific histone modifications.

Conclusion

Our understanding of epigenetic regulation in plants is rapidly growing. However, up to now, linkage of histone modifications, ATP-dependent chromatin remodeling, and DNA methylation to a specific stress and the origin of this specificity is still unknown. To identify targeted genes and regulatory complexes, genomic binding studies and proteomic analysis will be required, respectively. It is necessary to deepen our investigation of the epigenetic regulators in crops and their underlying molecular mechanism. Understanding the mechanism of epigenetic regulators and their regulatory networks in crops will be a potential tool for further exploitation toward sustainable agriculture. Moreover, it is desirable to design new breeding strategies in which the epigenetic variability should be taken into consideration. This seems even more realistic with the advancement of genomic technologies and cost lowering of next-generation sequencing. Like MAS (marker-assisted selection), epigenetic marker-assisted selection could also be initiated.

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